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COMPARISON OF AEROBICALLY-TREATED AND UNTREATED CROP RESIDUE AS A SOURCE OF RECYCLED NUTRIENTS IN A RECIRCULATING HYDROPONIC SYSTEM

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ABSTRACT

This study compared the growth of potato plants on nutrients recycled from inedible potato biomass. Plants were grown for 105 days in recirculating, thin-film hydroponic systems containing four separate nutrient solution treatments: 1) modified half-strength Hoagland's (control), 2) liquid effluent from a bioreactor containing inedible potato biomass, 3) filtered (0.2 µm) effluent, and 4) the water soluble fraction of inedible potato biomass (leachate). Approximately 50% of the total nutrient requirement in treatments 2 - 4 were provided (recycled) from the potato biomass. Leachate had an inhibitory effect on leaf conductance, photosynthetic rate, and growth (50% reduction in plant height and 60% reduction in tuber yield). Plants grown on bioreactor effluent (filtered or unfiltered) were similar to the control plants. These results indicated that rapidly degraded, water soluble organic material contained in the inedible biomass, i.e., material in leachate, brought about phytotoxicity in the hydroponic culture of potato. Recalcitrant, water soluble organic material accumulated in all nutrient recycling treatments (650% increase after 105 days), but no increase in rhizosphere microbial numbers was observed.

INTRODUCTION

In support of long-term human stays in space (i.e., the Moon or Mars), bioregenerative life support systems are being considered by NASA /1/. Based on human caloric needs, using higher plants for food production (carbon fixation) would also result in the plants supplying all of the crews' oxygen and potable water needs /2/. Closure of the oxygen and water loops would be fairly complete with this scenario. Crops in a bioregenerative system will also have inorganic nutrient requirements (i.e., fertilizer), so closure in this area would be beneficial in alleviating resupply costs. The ash (i.e., mineral) component of inedible plant tissue accounts for over 10% of dry mass from hydroponically grown crops /3, 4/. The solubility of nutrients from inedible biomass is relatively high. A low energy, water soak (leaching) of inedible wheat, soybean, and potato biomass has resulted in high recovery rates (> 50%) of most minerals, with the exception of calcium and iron /5/, and wheat grown with recycled nutrients from a leaching process has produced yields as great as plants grown with reagent-grade salts /6/.

Although leaching results in a high return of plant nutrients, about 25% of the organic portion of inedible biomass is soluble as well /5/. The soluble organics are quite labile and aerobic microbial degradation can reduce the total organic content (TOC) by more than 50% within a few days /7/. However, when using a recirculating nutrient delivery system and large additions of leachate, the TOC loading rates can outpace microbial degradation, resulting in TOC build-up and reduced plant growth /8/. Improving the TOC degradation rate would lower the solution TOC, which may result in better plant growth. A processing system that effectively reduces inedible biomass TOC can be incorporated into the production of other food types, such as fungal mycelia /7/. Secondary food production could indirectly improve a crop's calculated harvest index (edible dry mass ÷ total dry mass) /9/. Aerobic digestion of inedible biomass, via a bioreactor, has the potential of solublizing the inorganic nutrients, degrading the biomass, and creating a protein-rich, microbial-based food source to be used by secondary consumers, i.e., animals or humans. In this study we grew potato (a CELSS candidate crop) in recirculating hydroponic systems to test the performance of aerobically treated leachate (bioreactor effluent) as a means for nutrient recycling in a CELSS.

MATERIALS AND METHODS

Plant material used in the study consisted of 6-week-old nodal explants of potato (*Solanum tuberosum* L.) cv. Norland. Plants were grown in a controlled environment growth chamber using cool-white fluorescent lighting averaging 254 ± 43 µmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at canopy level, with a 12-h photoperiod. Temperatures were maintained at 19.9 ± 0.2 °C (light)/15.8 ± 0.2 °C (dark). Relative humidity was kept constant at 65 ± 1%. Carbon dioxide was added to the chamber atmosphere to provide approximately 100 ± 2 Pa partial pressure. Experimental design consisted of four nutrient tanks supporting two trapezoidal-shaped culture trays. Each 0.25 m^2 culture tray contained two plants (eight plants m^{-2}). Stainless steel, support cages (60-cm height) were placed around the perimeter of each culture tray to restrict plant growth to the allotted area. Fiberglass screening was wrapped around each cage and maintained at plant canopy level to reduce the effect of side lighting.

The specific protocol for leaching inedible plant biomass is described by Garland /5/. Briefly, the procedure involved soaking oven-dried biomass at a rate of 26 g biomass L^{-1} deionized water for a 2-hr duration and aerating the solution at 20 L min^{-1}. The leachate was then passed through a filter, and stored for use. The bioreactor used for treating biomass is described in detail elsewhere /10/. Bioreactor treatments involved loading oven-dried biomass at a rate of 16 g biomass L^{-1} deionized water, aerating the mixture at 15 L min^{-1}, with continuous, impeller-driven stirring, computer monitoring and control of pH, and monitoring of dissolved O_2. Biomass was added and harvested daily, and effluent separated from biomass via a coarse filter, and stored for use. Half of the effluent was filtered through a 2 micron filter, prior to storage.

Plants were grown hydroponically using recirculating nutrient film technique. Four different nutrient solutions were compared (Table 1):
A) modified, half-strength Hoaglands comprised solely of reagent-grade chemicals.
B) liquid fraction (effluent) from an aerobic bioreactor containing inedible potato biomass.
C) filtered (0.2 µm) effluent from an aerobic bioreactor containing inedible potato biomass.
D) the water soluble fraction (leachate) of inedible potato biomass.

Table 1. Nutrient concentrations of treatments prior to salt amendments.

Nutrient	Hoaglands (control)	Effluent	Filtered Effluent	Leachate
	mM	mM	mM	mM
NO_3-N	7.5	4.4	5.0	2.2
PO_4-P	0.5	0.2	0.2	0.4
K	3.0	2.7	2.7	3.0
Ca	2.5	0.3	0.4	0.0
Mg	1.0	0.4	0.4	0.4
	µM	µM	µM	µM
Fe	50.00	0.00	0.00	0.00
Mn	7.40	0.10	0.10	1.50
Zn	0.96	0.00	0.00	0.23
Cu	1.04	0.02	0.00	0.64
B	9.50	0.10	0.00	18.60
Mo	0.01	0.00	0.00	0.00

Table 2. Nutrient concentrations of replenishment concentrates prior to salt amendments.

Nutrient	Modified Hoaglands (control)	Effluent	Filtered Effluent	Leachate
	mM	mM	mM	mM
NO_3-N	62	42.0	42.0	55.0
PO_4-P	9	1.2	1.1	9.8
K	48	29.0	29.0	75.0
Ca	9	4.0	3.7	1.0
Mg	10	3.7	3.7	9.4
	µM	µM	µM	µM
Fe	134.0	12.0	11.0	101.0
Mn	67.0	12.0	13.0	39.0
Zn	8.6	0.0	0.0	5.8
Cu	9.4	2.0	10.0	16.0
B	86.0	84.0	92.0	464.0
Mo	0.1	0.0	0.0	0.0

Knowing the nutrient solutions (B - D) were not nutritionally complete, reagent-grade salts were used to make up the differences, where appropriate (Table 1). This resulted in the four nutrient solutions having comparable concentrations of the required elements. Subsequent nutrient replenishments that replaced plant uptake were calculated in a similar manner (Table 2). It is important to mention the harvest index of potato is approximately 80%, indicating that only about 20% of the nutrients should be supplied from the inedible portion of potato. However, it was decided to recycle a greater proportion of nutrients (50%), in order to exaggerate possible plant inhibitory effects from the increased organic carbon load.

Each nutrient solution was measured for electrical conductivity (EC) on a daily basis and nutrients replenished from a concentrate (Table 2) to maintain an EC between 0.10 - 0.13 S m^{-1}. Nutrient solution pH was automatically monitored and controlled at 5.8 with additions of 0.39 M nitric acid. Water uptake (from evapotranspiration) was replaced daily by manually adding deionized water to the reservoirs to maintain a constant volume of 20 L.

Nutrient solution pH, water replenishment, nutrient replenishment, and EC measurements were recorded daily. Weekly nutrient solution samples were taken from each reservoir for inorganic analysis using ICP and AA spectrometry methods. Monthly nutrient solution samples were analyzed for total organic carbon concentration using UV-assisted perfsulfate oxidation and total microbial cell density using the acridine-orange (AO) method with epiflourescent microscopy /11/ . The AO method was also used to estimate cell density in the rhizosphere. Suspensions of rhizosphere organisms suitable for microscopy were prepared by shaking excised root samples for two minutes in 0.1% sodium pyrophosphate solution containing glass beads. Diurnal photosynthetic rates were measured at 54 days after planting (DAP) using a LI-COR 6200 leaf cuvette system. At 105 DAP, all plants were harvested, separated into tops, roots, and tubers (< 2.5 cm and > 2.5 cm), oven-dried for 3 days at 70 °C, and weighed. Subsamples of all tissue were ground through a 2-mm mesh screen in a Wiley mill and shipped to Dr. Wade Berry (University of California, LosAngeles, CA, USA) for inorganic analysis.

RESULTS AND DISCUSSION

Plants grown in the bioreactor effluent (either filtered or unfiltered) had similar dry mass values as those grown in the Hoagland's (control) solution (Table 3). However, the leachate treatment had shorter plants, with fewer main stem nodes (Table 3), resulting in shorter internode lengths. Tuber and total dry mass were also less with the leachate treatment, but tuber dry mass was not significantly different from the control (Table 3) (note that the CV was 21% for tuber dry mass on a per plant basis). Harvest index (tuber dry mass:total dry mass) was slightly higher in leachate plants because of less vegetative biomass, although differences were not significant (Table 3). Based on the harvest data, only the leachate treatment had a detrimental effect on potato growth.

Upper canopy single leaf photosynthetic (CO_2 exchange) rates averaged about 12 µmol m^{-2} s^{-1} for control and bioreactor treated plants, and 10 µmol m^{-2} s^{-1} for leachate treated plants (data not shown). The slight depression in plants from the leachate treatment may have been associated with plant stress, which is known to reduce photosynthesis /13/. Dark-period respiration was similar among all treatments, averaging about 1.0 µmol m^{-2} s^{-1} (data not shown).

Total organic carbon (TOC) accumulated in the nutrient solution of all the recycling treatments, increasing from a concentration of 2 mM at day 20 to approximately 10 mM by day 56, where as levels remained below 0.5 mM in the Hoagland's solution throughout the growout (Fig. 1a). The total amount of organic material added to the leachate treatment was five times greater than that added to the effluent treatments, as calculated by multiplying the volume of replenishment solutions added per day to each system by the organic content of the respective replenishment solution treatments (Fig. 1b). This additional carbon was apparently degraded by the microbial communities associated with the rhizosphere, nutrient solution, and /or biofilms within the leachate-based systems. The recalcitrant dissolved organic material that accumulated in all the recycling treatments did not appear to negatively affect plant growth, as evidenced by the high yields in the effluent-based systems.

The reduced yields in leachate-based systems suggests that the addition of the labile organic fraction of inedible potato biomass either directly or indirectly impaired plant growth. Previous short-term plant growth studies have shown that potentially phytotoxic compounds are present in this carbon pool /8/.

Table 3. Effect of different nutrient recovery treatments on growth of potato.

Treatment	Height	Main stem nodes	Tuber dry mass	Total dry mass	Harvest Index*
	mm	No. plant^{-1}	g plant^{-1}	g plant^{-1}	(%)
Hoagland's (control)	770	29	160	217	74
Effluent	885	29	167	226	74
Filtered Effluent	818	30	172	235	74
Leachate	441	21	101	133	77
LSD (0.05)	124	5	63	79	8
CV (%)	9	10	21	20	6

** Harvest Index = (Total plant dry mass ÷ tuber dry mass) x 100.*
Least significant differences (LSD) were determined using Dunnett's two-tailed t-test, where n = 4.

Potential indirect effects include changes in the density or composition of the microbial communities associated with the plant growth systems, and/or interactions with inorganic elements leading to nutrient toxicity or deficiency. Microbial density was not increased in either the rhizosphere or nutrient solution of the leachate-based systems (Fig. 2). These results are similar to those reported in leachate-based and Hoagland's based systems used to grow wheat /6/. The lack of any negative effect on plant growth of the unfiltered bioreactor effluent, despite consistently high suspended cell counts in this treatment, suggest that the microbial community associated with degradation of labile organics did not contain phytopathogenic or other plant growth inhibiting bacteria.

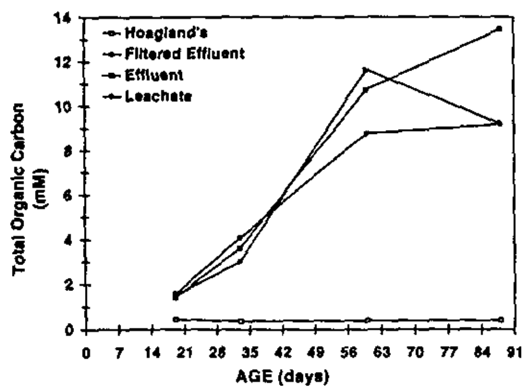

Figure 1a. Mineral recycling treatment effect on total organic carbon content of nutrient solutions over time.

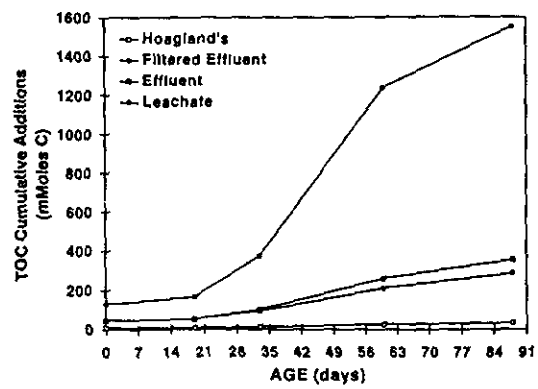

Figure 1b. Mineral recycling treatment effect on total organic carbon additions into nutrient solutions over time.

The inorganic nutrient content of plant tissue (tops (leaves + stems), tubers, and roots) for each nutrient treatment was analyzed to determine potential nutrient toxicities or deficiencies. All micronutrient concentrations were within acceptable ranges (data not shown). Macronutrient concentrations in plants from recycling treatments relative to those in plants from the Hoagland's treatment are shown in Table 4. Plants from the leachate treatment had a relatively low allocation of P and Mg in vegetative portions. The P levels were below critical values for plant health /13/ and may have been the cause of some visual symptoms (i.e., poorly developed, stiff leaves) in our study /14/. Tuber calcium levels were lower in all the recycling treatments, and were below critical levels in the leachate treatment /13,15/. However, there were no signs of tuber defects associated with Ca deficiencies, such as internal brown spot /16/.

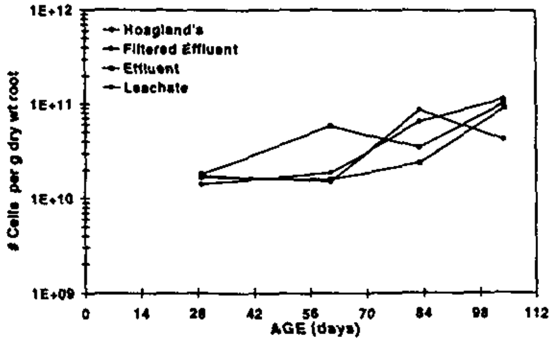

Figure 2a. Mineral recycling treatment effect on microbial number per gram dwt. of potato root.

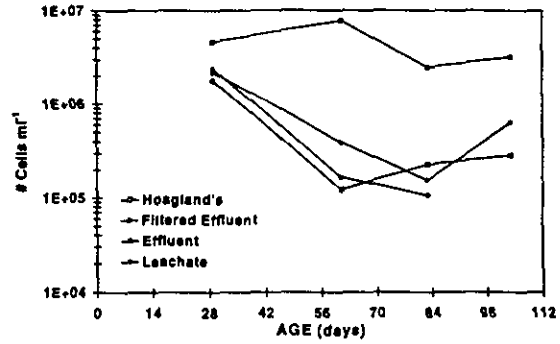

Figure 2b. Mineral recycling treatment effect on microbial number per ml of nutrient solution.

Table 4. Comparison of tissue nutrient concentrations from recycling treatments as a percentage of Hoagland's controls. Tissue was analyzed at harvest (105 days).

TREATMENT	P	K	Ca	Mg	Fe
TOPS*					
Hoagland's	2992 ppm	90815 ppm	15778 ppm	6368 ppm	153 ppm
	(% of control)	(% of control)	(% of control)	(% of control)	(% of control)
Effluent	107	108	101	106	115
Filtered effluent	100	97	105	106	108
Leachate	50	91	107	76	90
TUBERS					
Hoagland's	2492 ppm	30463 ppm	1322 ppm	2717 ppm	151 ppm
	(% of control)	(% of control)	(% of control)	(% of control)	(% of control)
Effluent	100	99	74	99	150
Filtered effluent	97	93	66	94	92
Leachate	71	89	11	90	76
ROOTS					
Hoagland's	9389 ppm	42229 ppm	20346 ppm	8490 ppm	2032 ppm
	(% of control)	(% of control)	(% of control)	(% of control)	(% of control)
Effluent	76	80	80	94	69
Filtered effluent	50	70	74	100	36
Leachate	67	99	70	50	68

**Tops defined as leaves + stems.*

Analysis of the tissue nutrient concentrations indicates that P deficiency may have been a potential cause for the decreased plant growth in the leachate treatment. A few reports have shown reduced phosphorous uptake of plants grown on phenolic acids or other specific allelopathic chemicals /17, 18/. Although the concentration of PO_4-P in the leachate replenishment solution was equivalent to that in all the other treatments, the P concentration in the nutrient solution remained lower than in the other treatments (data not shown). A lower phosphorous recovery (i.e., amount recovered in biomass ÷ amount added to solution) occurred with the leachate treatment. Recovery losses of P and Ca have been seen in many of our recirculating hydroponic studies, even when using reagent-grade salts. This information suggests the leachate-based systems may have contained a sink for P significant enough limit to P availability to plants. Chemical precipitates were not visible in this, or any of the other systems. However, the leachate treatment had a noticeable film on the nutrient delivery system surfaces, which was less apparent in the effluent treatment, and even less so in the filtered-effluent treatment. Chemical or microbiological analysis of these biofilms were not performed, but greater biofilm accumulation in response to increased organic loading from leachate treatments has been previously reported /6/. Biofilms are known to adsorb, entrap, and/or precipitate inorganic material /19/. Use of nutrient solution mineral activity and complexation models, such as VEGACT /20/, could be a useful aid in determining nutrient recoveries in hydroponic solutions. These models compute complexation with other species, as well as characterize possible precipitate formations in the nutrient solutions. Obtaining a better understanding of nutrient solutions and thus an improved accountability of the various nutrients in the system is necessary for making sound comparisons of nutrient recycling methods in a CELSS.

CONCLUSIONS

Approximately 50% of the total nutrient requirement of hydroponically-grown potatoes was recycled using the water soluble fraction of inedible potato biomass. Microbial degradation of labile organic material within this fraction, via aerobic bioreactors, eliminated the growth inhibition observed with direct use of leachate. The growth-inhibitory effects of the labile organic material could be due to direct phytotoxic interactions associated with changes in the microbial community, or interactions with inorganic nutrition. The data did not support any role of microorganisms relating to decreased plant growth. Microbial densities were unaffected in the leachate treatment and adequate plant growth in the unfiltered bioreactor effluent indicated that microorganisms responsible for degradation of the organic material were not deleterious to plant growth. Phosphorus levels in the vegetative biomass of the leachate treatment were deficient despite equivalent additions of phosphate to the systems, suggesting lower uptake and/or a potential phosphorous sink. Future studies will concentrate on evaluating potential complexation of P with other elements as well as absorption to biofilms on hardware surfaces, in order to better understand nutrient budgets in recycling treatments.

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